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NOXYACETIC ACID IN SOIL IN RELA-
TION TO CONTENT OF WATER,
ORGANIC MATTER, AND LIME

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY

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By
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PERSISTENCE OF 2,4-DICHLOROPHENOXYACETIC ACID IN SOIL IN RELATION TO CONTENT OF WATER, ORGANIC MATTER, AND LIME¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 583

OLIVE H. KRIES

Introduction

The increasing use of 2,4-dichlorophenoxyacetic acid (2,4-D) in spray applications to plants for various purposes and the possibilities in expanding its use to include direct treatment of soil necessitate the investigation of its possible effects upon and persistence in soils of diverse types in relation to various management practices. Experiments reported upon to date (1, 4, 5, 6, 7, 8, 11, 12) indicate that the length of persistence in soil of the toxic effects to higher plants varies with soil type and management as well as with the amount applied, the method of application, and the kind of plant.

The present experiment was devised to secure additional data on the persistence of activity of 2,4-D in one soil in relation to its content of water, organic matter, and lime. It was carried on in the University of Chicago greenhouses from March 27 to August 31, 1946.

Material and methods

Heavy black loam from northern Illinois was mixed with coarse sand in the

proportion of 3:1 by volume. Approximately 1440 pounds of the mixture were divided into six lots, two of which were allowed to become air-dry; the other four lots were kept moist. Prior to drying, partially decomposed leaf mold was added to one of the lots to be air-dried and to two of the moist lots in the ratio of 1:3 by volume. Lime (CaCO_3), at the rate of 1 lb. to 500 lb. of soil, was mixed with two of the four moist lots on March 27. The six lots thus included: dry soil; dry soil + leaf mold; moist soil; moist soil + leaf mold; moist soil + lime; and moist soil + lime + leaf mold. These terms will be used in referring to these lots hereafter.

After preparation all six lots were stored in large boxes in a room adjacent to the greenhouse. The reactions of the various combinations ranged from pH 7.8 to 8.2; moisture equivalents, determined by the centrifuge method, from 19.2% to 21.5%. Water content in the moist lots ranged from 21.5% to 24.3% during the first week of storage.

Meanwhile, 36.0-, 3.6-, and 0.36-gm. quantities of commercial grade 2,4-D were each dissolved in 100 ml. of ethyl alcohol. The three solutions were each then thoroughly mixed by hand with

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1-kg. amounts of white quartz sand which was spread out to allow evaporation of the alcohol.

The six different lots of stored soil were each then divided into four aliquots. To three aliquots of each lot, the dry sand containing 2,4-D was added on April 7 (11 days after the lime had been added) so as uniformly to incorporate 2,4-D into the soil at the rate of 2, 20, and 200 p.p.m., respectively, based on the dry weight of the soil. The fourth aliquot of each lot served as an untreated control. The amount of sand so added was negligible in its effect upon textural and structural relations of the soil.

The twenty-four lots of soil which resulted from the possible combinations of the various treatments were placed in storage in a room not subject to great temperature fluctuations; temperatures 2-4 inches below the surface in the stored soil averaged about 74° F. with a low of 58° on April 26 and a high of 84° on July 8.

Water was added to the moist lots occasionally to offset evaporational losses but not in such quantity as to result in leaching.

On April 14 and 21, May 5, July 17, and August 15, soil from each lot was placed in 5-inch, white, glazed pots provided with glass-wool filters over the drainage hole. Torpedo sand filled the lower one-fourth of each pot. On April 14 and 21 ten pots were so filled from each of the twenty-four stored lots, and on the other dates five pots were filled from each. Thus, soil was taken from each storage lot into the greenhouse 1 week, 2 weeks, 4 weeks, 14½ weeks, and approximately 18 weeks after 2,4-D had been mixed with it.

On each removal date the filled pots were placed on a greenhouse bench, and each pot was uniformly planted with five Red Kidney beans and ten seeds each of

barley, African marigold, and white mustard. These species are sensitive in different degree to 2,4-D. The rate and percentage of emergence of the seedlings were used as primary criteria of the degree of toxicity of 2,4-D and of its persistence in the different stored lots on the various planting dates.

Half the number of pots in each treatment in the April 14 planting were replanted on May 12. In this process the soil was allowed to dry to the point where the old plants could be pulled free with little loss of soil. The other pots in the April 14 planting were replanted on June 2. A third planting was made on July 15 on the soil initially planted on April 14 and replanted on May 12.

Similarly, half the number of pots of the April 21 sowing were replanted on May 19. In the cases of two pots of each of the twenty-four treatments, the top and bottom halves of the soil mass were removed to clean, separate pots to test whether residual toxicity differed in the top and bottom layers of the soil.

On August 16 the pots initially planted on July 17 were replanted.

Ten days after each planting all but one of the bean seedlings in the control pots were cut off below the cotyledons so that the smaller seedlings would not be excessively shaded. This procedure was not necessary in the first plantings in the pots containing 2,4-D because of its inhibiting effect on beans.

Counts of germination were made 14 days after each planting. Germination was considered, following PORTER (9), as the emergence and development from the embryo of those essential structures which, for the kind of plant in question, were indicative of the ability to produce a normal plant under favorable conditions. All such data were calculated in percentages and graphed (figs. 1-4). Further development of the plants was fol-



FIG. 1.—Percentages of emergence of test plants 14 days after planting on indicated dates from soil which had been stored since April 7. Conditions of storage were: *A*, dry soil; *B*, dry soil + leaf mold; *C*, moist soil; *D*, moist soil + leaf mold; *E*, moist soil + lime; *F*, moist soil + lime + leaf mold. Each group of four bars, from left to right, respectively, represents emergence from soil in which 2,4-D had been incorporated on April 7 at rate of 0, 2, 20, and 200 p.p.m.



FIG. 2.—As in fig. 1 for other test species

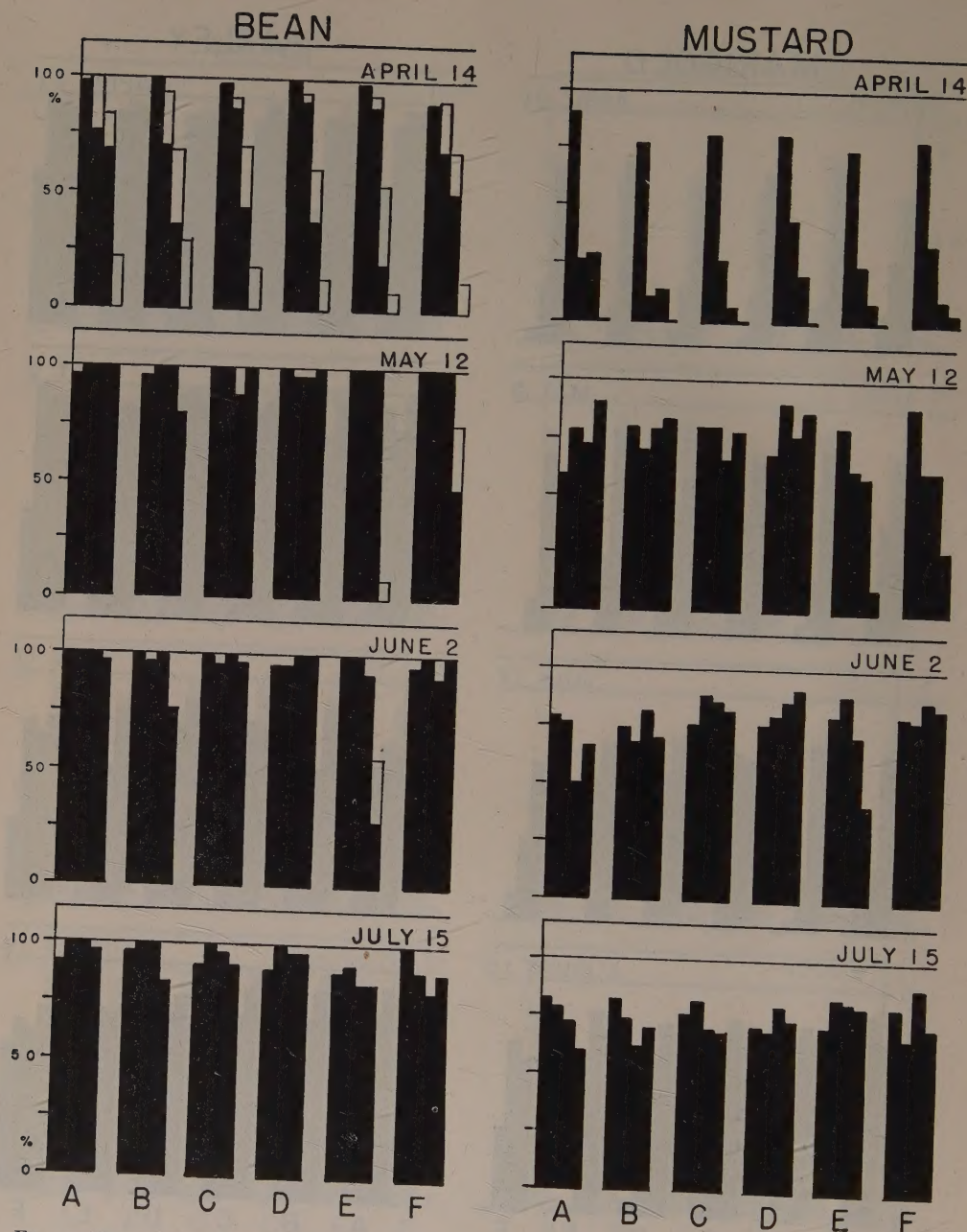


FIG. 3.—Percentages of emergence of test plants 14 days after planting on indicated dates. Each group of four bars and categories A, B, C, D, E, and F as in fig. 1. Planting on April 14 made after 1 week of soil storage. Data for other dates are of replantings on same soil following 4, 7, and 13½ weeks of cropping. Note effect of lime (E) on retention of toxicity and of leaf mold (F) in mitigating its effect.

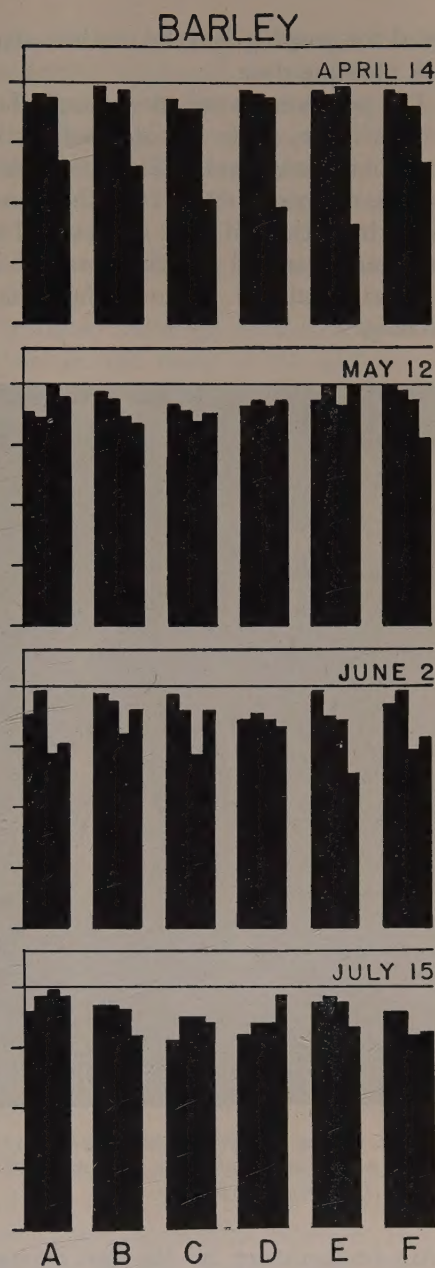
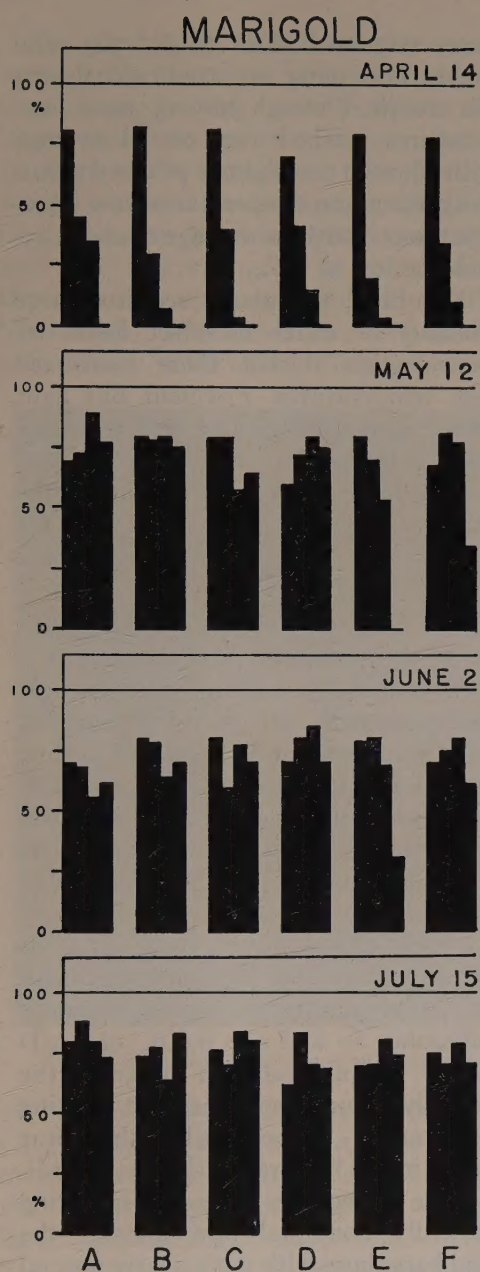


FIG. 4.—As in fig. 3 for other test species

lowed for varying lengths of time after each planting date.

The pots were watered sparingly but frequently so as to avoid leaching of 2,4-D in drainage water. Thus, the eventual loss of toxicity either from the stored soil or from the soil after its removal to the greenhouse and planting was due in part, at least, to factors other than leaching.



FIG. 5.—Beans 26 days after planting on May 5 in moist soil + lime. *Left*, from 2-p.p.m. (2,4-D) pot; *right*, from 20-p.p.m. pot.

Air temperature in the greenhouse from April 21 until the last week in May varied from 90° to 98° F. at midday to a night minimum of about 74°. During the last week in May and practically all of June, daily maximum readings stood at 98° with one exception. During the first part of the third week of June, tempera-

tures remained close to 80° day and night. A low of 65° occurred twice during the month. Through July 15, noon temperatures reached 100° on an average with a low on one night of 76°. In August, daily maximum temperatures were somewhat lower, with an average of about 95° and one low of 74°.

Naturally, the plants required large amounts of water to offset losses by transpiration during these continued high temperatures. Frequent but light watering was maintained. Soil temperatures in the pots at a depth of 4 inches ranged from 59° to 98° F. during the season.

Results

SOME EFFECTS OF 2,4-D ON SEEDLING DEVELOPMENT

As judged by seedling emergence in this experiment, mustard and marigold seeds were more intolerant of 2,4-D than Red Kidney bean and barley, and marigold seemed to be more sensitive than mustard. The sensitivity of marigold to 2,4-D has been previously reported (2, 3).

Development of bean seedlings deviated from normal to such an extent in all the initial plantings on soils originally containing 20 and 200 p.p.m. of 2,4-D that it was necessary to designate the seriously injured specimens in making the counts. Consequently, the clear spaces in the bar graphs (figs. 1, 3) indicate the percentages of seedlings showing markedly abnormal development, the solid bars those with more nearly normal development. The seriously injured seedlings were often upside down and much contorted (fig. 5). Frequently, they possessed four flanges of tissue at the bases of the hypocotyls. These abnormalities have been described by others (1, 4).

The capacity of substituted phenoxy-acetic acids to induce modifications in size, shape, pattern, and venation of leaves was first reported in 1942 (13). In the present experiment the heart-shaped leaves of beans strongly affected by 2,4-D often failed to expand properly and were yellowish-green in color with the veins more or less outlined with small black dots. In general, they appeared stiff and dry. Some of the trifoliate leaves

in any later plantings although the irregularities appeared in some of the 2,4-D treatments in all the successive initial plantings on stored soil.

Mustard germinated first in each planting, and those mustard seedlings which emerged from soil treated with 2,4-D exhibited no characteristic abnormalities.

Marigold seedlings developed swollen regions at the bases of the petioles in



FIG. 6.—First "trifoliate" leaves of beans from various 20-p.p.m. (2,4-D) pots in April 14 planting

of bean displayed teleomorphic effects (2, 3) from 2,4-D in the production of excessive numbers of leaflets as well as in the nondivergence of leaflets. Some leaves had one leaflet only, while others had as many as six or eight divisions (figs. 5, 6). No specimens were found with more than ten leaflets per leaf. Judging from counts made on these anomalous leaves of bean, more plants in the first sowing (April 14) in the soil treated with 2 and 20 p.p.m. of 2,4-D possessed this type of malformation than

some plants in the initial plantings in soil originally containing 20 p.p.m. of 2,4-D. Occasionally, the leaves seemed narrower than those in the controls. If germination was possible, however, there were no striking morphogenic effects from 2,4-D on either marigold or mustard.

In barley the coleoptiles remained very pale, almost white, for several days in the initial plantings in soil originally containing 200 p.p.m. of 2,4-D. Later, the green plumules emerged weakly and elongated

a few inches. A brown spotting superficially resembling a rust developed on the leaves of plants in the 2- and 20-p.p.m. soils in most of the initial sowings in stored soil. Frequently it was followed by a chlorosis of the entire leaf. A burning of the leaf tip was also rather common in these pots.

GERMINATION IN CONTROL POTS

Some deviations in the percentages of germination in the controls were apparent in relation to season of planting, but the six major soil categories had little effect on the percentages of emergence in control pots on any one planting date. Throughout the experiment Red Kidney bean was high in percentage of germination in all controls. The percentage of germination for mustard was noticeably reduced in the July 17 planting, while the percentage for barley was relatively low in both the July 17 and the August 15 plantings. On the whole, of the four species tested, marigold was lowest in percentages of germination in controls for all ten planting dates. All statements concerning the effects of 2,4-D upon emergence are based on comparisons with the control pots in the same category of soil.

INHIBITORY EFFECTS OF 2,4-D IN STORED SOIL

The persistence of the toxic effect of 2,4-D in stored soil is shown in table 1, in figures 1 and 2, and in the data for April 14 in figures 3 and 4, based chiefly on percentages of emergence of test species in the five different plantings on stored soil. The data in table 1 are weighted somewhat in accord with the later development of the seedlings.

Results from soil stored 1 week (figs. 3-4 for April 14) and from soil stored 2 weeks (figs. 1-2 for April 21) were

practically identical. Toxicity to bean, marigold, and mustard was apparent at all three levels of concentration of 2,4-D in all six categories of soil treatment. For barley there were no inhibitory effects in the soil originally containing 2 and 20 p.p.m. of 2,4-D in any of the six combinations.

Results from the planting on May 5 (figs. 1-2), after 4 weeks of soil storage, were much the same as in the previous plantings, except that Red Kidney bean showed little or no injury in the 2-p.p.m. soil, and barley appeared to be stimulated in the 20-p.p.m. dry-soil treatment.

In the July 17 planting on soil stored for 14½ weeks, there were no pronounced inhibitory effects on any of the four species in the moist-soil lots at the 2- and 20-p.p.m. levels. All the seeds in the moist-soil lot at 200 p.p.m. were affected, however. The other five major soil categories showed toxicity essentially the same as in the three earlier initial plantings on stored soil.

In the last of the initial sowings on August 15, on soil stored 130 days, pronounced inhibitory effects continued with some exceptions: the lots of moist soil were least toxic of all, and moist soil+leaf mold, with or without added lime, was less toxic than the other three soil combinations.

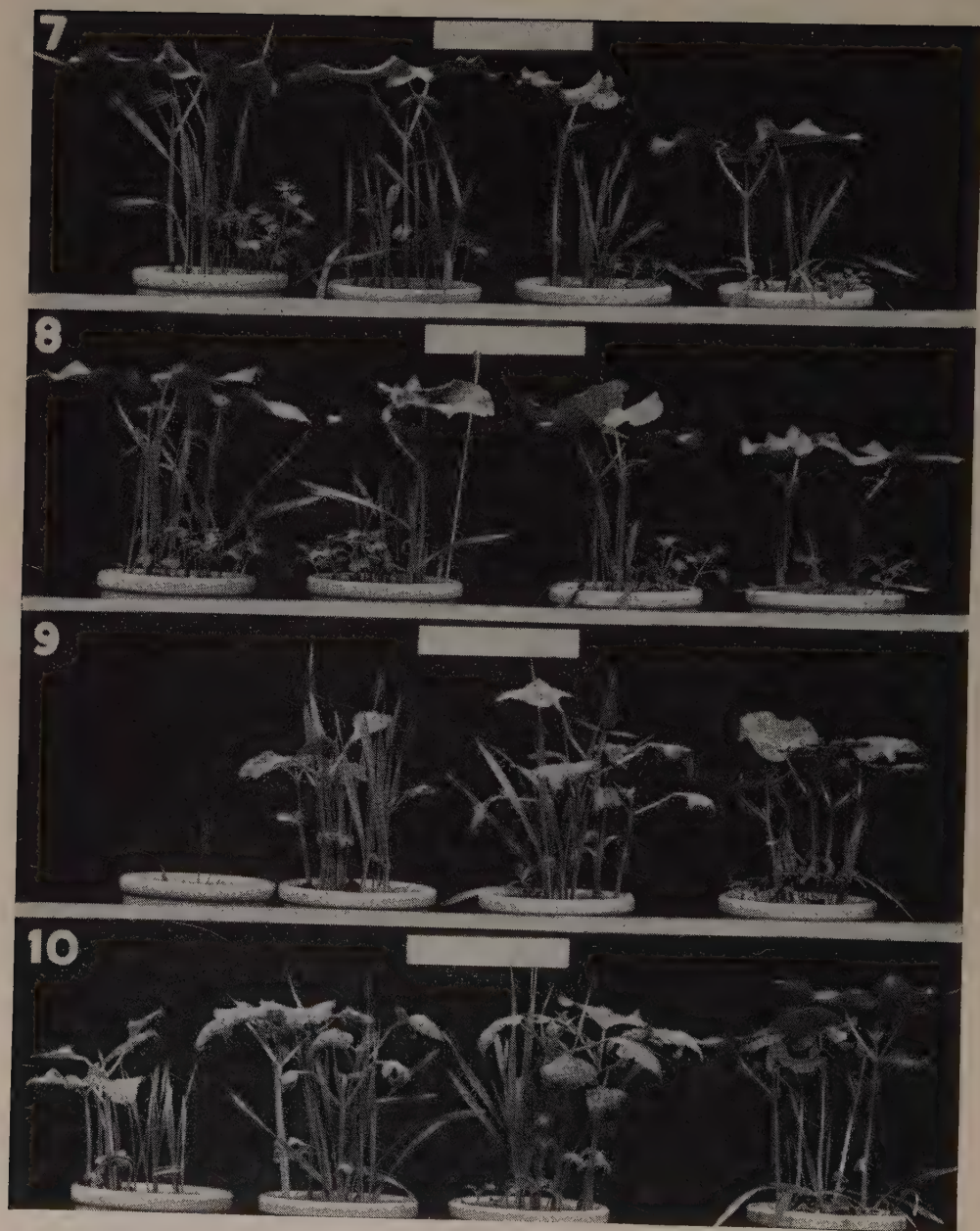
Thus, in general, there was very slight difference among soil lots stored for 1, 2, or 4 weeks in the persistence and degree of toxicity of equivalent amounts of 2,4-D added at the beginning of storage. The persistence was little affected by moisture content of soil, by the status of its lime content, or by the addition of leaf mold. After 14½ weeks of storage, however, there had been a pronounced loss of toxicity in the moist-soil lots which contrasted strongly with the five other categories. There was a gradual

TABLE 1

PRESENCE OR ABSENCE OF TOXIC OR STIMULATING EFFECTS WHICH RESULTED FROM MIXING 2,4-D IN SOIL ON APRIL 7
AS SHOWN BY SEEDLING EMERGENCE AND LATER DEVELOPMENT OF FOUR TEST SPECIES*

INITIAL SOIL	2,4-D ADDED (P.P.M.)	SOIL IN STORAGE APRIL 7-14; THEN IN GREENHOUSE				SOIL IN STORAGE APRIL 7-21; THEN IN GREENHOUSE		SOIL IN STORAGE APRIL 7- MAY 5; THEN IN GREENHOUSE	SOIL IN STORAGE APRIL 7-JULY 17; THEN IN GREENHOUSE		SOIL IN STORAGE APRIL 7- AUGUST 15; THEN IN GREENHOUSE
		Planted April 14	Replanted May 12	Replanted June 2	Replanted July 15	Planted April 21	Replanted May 19	Planted May 5	Planted July 17	Replanted August 16	Planted August 15
Dry soil.....	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	-- SS --	-- -- --	S S-- S--	++ ++ ++	-- S-- +	++ ++ ++	++ ++ ++	-- ++ ++	++ ++ ++
Dry soil+leaf mold.....	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	-- -- --	-- -- --	S S-- S--	++ ++ ++	-- -- ++	++ ++ ++	++ ++ ++	-- ++ ++	++ ++ ++
Moist soil.....	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	-- -- --	-- -- --	S -- --	++ ++ ++	-- -- --	++ ++ ++	-- -- ++	-- -- ++	-- -- ++
Moist soil+leaf mold.....	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	-- S S	-- S --	S S-- S--	++ ++ ++	-- S S	++ ++ ++	++ ++ ++	-- -- ++	++ ++ ++
Moist soil+lime.....	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	++ ++ ++	-- ++ ++	-- S --	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	-- ++ ++	++ ++ ++
Moist soil+lime+leaf mold.	$\begin{Bmatrix} 2 \\ 20 \\ 200 \end{Bmatrix}$	++ ++ ++	++ ++ ++	-- ++ ++	-- S --	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	-- ++ ++	++ ++ ++

* From left to right, each group of entries indicates effect on bean, mustard, marigold, and barley, respectively; "+" indicates obvious toxicity; "S" shows obvious stimulation; "--" indicates no effect. Data are based on comparison with plants in control soil similarly treated except lacking 2,4-D.



FIGS. 7-10.—Test plants on May 25 in replanting of May 12. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 7, moist soil; fig. 8, moist soil + leaf mold; fig. 9, moist soil + lime; fig. 10, moist soil + lime + leaf mold.

diminution of toxicity in the lots of moist soil+lime+leaf mold as shown by the plantings of May 5, July 17, and August 15 in contrast with strong persistence of toxicity in the moist soil+lime combination.

INHIBITORY EFFECTS IN REPLANTED SOIL

As shown by the replanting on May 12 in soil which had been cropped in the

however, had markedly reduced the toxic condition in the limed soil, especially in the case of marigold, and for bean in the 200-p.p.m. soil (figs. 9, 10). Some apparent stimulation of growth was evident in mustard, barley, and bean in the lots of dry soil with initial concentrations of 20 and 200 p.p.m. of 2,4-D, and also in mustard and marigold in all levels in moist soil+leaf mold (fig. 8).

A replanting on June 2 of soil which



FIGS. 11-13.—Test plants on May 25 in replanting of May 19. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 11, moist soil + lime; fig. 12, top half of soil mass in planting of April 21, moist soil + leaf mold; fig. 13, bottom half of soil mass.

greenhouse for 4 weeks, toxic effects from 2,4-D had completely disappeared except in the limed lots of soil (figs. 7-10). In the latter, some toxicity to one or more of the test species was still present at all test levels of 2,4-D (table 1, figs. 3, 4), although generally in lower degree than in the April 14 planting. The presence of partially decomposed leaf mold,

had been cropped in the greenhouse for 7 weeks showed strong inhibition in the 200-p.p.m. moist soil+lime, except in the case of barley, although the toxicity was somewhat less than on May 12. As in the May 12 replanting, the presence of leaf mold had further reduced the toxic condition in the limed soil.

In the July 15 replanting in soil which

had been cropped in the greenhouse for $13\frac{1}{2}$ weeks, toxic effects from 2,4-D had completely disappeared in all treatments, including the limed soils. Moreover, there was some apparent stimulation to growth of all test species, in comparison with controls, in some of the lots which had been treated with 2,4-D (table 1).

In comparing the replantings of May 19 and August 16 with the replanting of May 12 (table 1), all three of which were made on soil which had been cropped in the greenhouse for 4 weeks, it appeared



FIG. 14.—Test plants on June 10 in replanting of May 19 in soil originally planted on April 21, moist soil + lime + leaf mold. *Left*, bottom half of soil mass in April 21 planting; *right*, top half.

that the May 19 replanting practically repeated the results of May 12, except in the case of dry soil+leaf mold, which remained toxic to the three dicotyledons in the 200-p.p.m. soil. In the resowing of August 16, deleterious effects of 2,4-D had apparently been more persistent than in the other comparable replantings, since there was still pronounced toxicity to one or more test species in all soil originally containing 200 p.p.m. of 2,4-D. Possibly the very high greenhouse temperatures during the August 16 test intensified the activity of plants in response to the presence of the growth-regulator, or for various reasons it had

not been lost from the soil or inactivated as rapidly.

In the replanting on May 19 of the upper and lower portions of soil from pots which had been cropped in the greenhouse for 4 weeks, a strong contrast in residual toxicity between top and bottom portions, as shown by seedling emergence and later development, occurred only in the 200-p.p.m. lots (figs. 11-14). The lower half was definitely more toxic to mustard and marigold, but Red Kidney bean in this portion also showed symptoms of toxicity in misshapen leaves and yellowish-green color in the seedlings. The effect of leaf mold in diminishing the toxicity of 2,4-D in the limed soil was well shown in this planting (figs. 11, 14).

These data suggest that some of the relatively greater decrease in toxicity of the top layers may have resulted from the downward leaching of 2,4-D from the soil in the pots, in spite of the care used in watering, especially since it disappeared much less rapidly from moist stored soil. HANKS (6) and DE ROSE (4) have both shown that 2,4-D may be leached rapidly from some soils.

In conclusion, in soil lots lacking added lime, 4 weeks of cropping in the greenhouse generally sufficed to eliminate the toxicity of all the different original levels of 2,4-D. There was, however, strong residual toxicity in the limed lots in all three replantings which followed 4 weeks of cropping. Some toxicity in the 200-p.p.m. treatment persisted in the limed soil after 7 weeks of cropping, and possibly more, but disappeared completely between the replanting dates of June 2 and July 15. In each replanting test the presence of partially decomposed leaf mold had offset, to a considerable extent, the effects of the added lime in delaying the loss of

toxicity, especially in the 2- and 20-p.p.m. levels.

Discussion

The striking persistence, after cropping for 4 weeks, of the toxic effects of 2,4-D on mustard and marigold in the limed soil originally treated with 2, 20, and 200 p.p.m. of 2,4-D, and on Red Kidney bean at the 200-p.p.m. level, is regarded by the writer as the most significant result of the experiment. In an experiment on leaching 2,4-D from soil, HANKS (6) found that its inhibitory effects persisted longer in a naturally alkaline, sandy clay soil, pH 8.4, than in any other of the six soils tested. He found, however, that addition of lime to these six soils seemed to produce no significant differences in toxicity of comparable leachates as tested by a slight modification of the corn-root bio-assay developed by SWANSON (10). In confirming the results of the bio-assay, HANKS tested Red Kidney bean emergence and development and observed that the alkaline soil mentioned was the only one showing toxic effects of 2,4-D to bean at the end of 6 weeks of leaching. HANKS's experiment was based on an original 20-p.p.m. level of 2,4-D mixed in the soil in contrast to the concentrations of 2, 20, and 200 p.p.m. used in the writer's experiment.

TAYLOR (11), working with two soils, pH 7.8-7.9 and pH 6.9-7.0, found that dicotyledonous weeds were almost completely eliminated for the growing season on these medium-light field soils treated at the start of the season with 1-4 lb. of 2,4-D per acre. In general, he found that the development of bean and mustard was more strongly inhibited than that of wheat and corn in proportion to intensity of 2,4-D treatment. He further noted the possible influence of marl (CaCO_3) in

the soil which was low in organic matter. He reported a persistence as long as 7 weeks of the toxic effects of 2,4-D applied at the rate of 4 lb. per acre. In the greenhouse the writer found that effects of 2,4-D at an initial concentration of 200 p.p.m. continued for 50 days, at least, in the case of the moist soil+lime.

HAMNER *et al.* (5), working with muck soil, reported that the toxic effects of 2,4-D mixed in it did not persist longer than 4 weeks. HANKS (6) found that the leachate from peat so treated was relatively free of toxicity, as measured by corn-root growth, 2 weeks after the incorporation of 2,4-D. In the writer's experiment the presence of large amounts of leaf mold in the soil reduced the effect of added lime in retarding the loss in toxicity.

After 18½ weeks treated soil in storage had retained most of its toxicity at all levels of 2,4-D with one exception, that of moist soil with original concentrations of 2 and 20 p.p.m. of 2,4-D. There was practically no loss of toxicity in soils stored air-dry. MITCHELL and MARTH (7) found a retention of toxicity for as long as 18 months in soil in relatively dry storage.

The more vigorous growth of some seedlings in 2,4-D-treated soil, in contrast to controls, in the replantings of May 12, June 2, and July 15 is noteworthy in many of the soil combinations. This apparent stimulation was greatest in the mixture of moist soil+leaf mold. HANKS found a stimulation of corn-root growth, in contrast with controls, in one or more of the weekly leachates from five of six soils treated with 2,4-D, the exception being the naturally alkaline soil mentioned previously. TAYLOR (11) also reported a stimulating effect on some plantings on soil previously treated with 2,4-D.

It has been demonstrated (1, 4) that germinating seeds are very susceptible to small amounts of certain growth-regulating substances, certain broadleaf species being more sensitive than some cereals. The behavior of Red Kidney bean, African marigold, and mustard in this experiment paralleled these findings for the dicotyledons, while barley showed resistance to 2,4-D in all initial plantings at 2 and 20 p.p.m. The single exception to this statement concerning barley appeared in the planting of July 17, especially on moist soil+lime, in which higher temperatures may have been an important factor in accentuating the toxic symptoms.

Modification of leaf form in bean repeated itself with surprising regularity in initial plantings in the 20-p.p.m. pots and resembled somewhat the morphogenic effects on various leaves described by ZIMMERMAN and HITCHCOCK (13) and by DE ROSE (4). The appearance of Red Kidney bean seedlings in the initial plantings on soil containing 2, 20, and 200 p.p.m. of 2,4-D in this experiment closely duplicated those illustrated by ALLARD *et al.* (1).

Leaching, decomposition through action of micro-organisms in the soil, and inactivation due to adsorption or fixation by soil colloids have been suggested as factors contributing to the reduction or loss in activity of 2,4-D in soil (4, 6). The writer's experiment suggests that the status of its lime content and the organic content of soil may strongly affect the rate of loss of toxicity from 2,4-D mixed in the soil, whatever the mechanisms involved. Toxicity was also gradually diminished under some conditions of moist storage of soil in contrast with its retention in dry storage, although conditions in moist cropped soil led to a much more rapid loss of toxicity than did those

in comparable moist stored soil. It is thought that reduction of toxicity in stored soil could not have resulted from leaching and that leaching was not the primary factor in the loss of toxicity in cropped soil.

Summary

1. Partially decomposed leaf mold, water, and lime were mixed with heavy black-loam soil from northern Illinois in such fashion as to yield six lots: air-dry soil; air-dry soil+leaf mold; moist soil; moist soil+leaf mold; moist soil+lime; and moist soil+lime+leaf mold. To four equal aliquots of each of the six lots, 2,4-dichlorophenoxyacetic acid (2,4-D) was mixed on April 7 at the rate of 0, 2, 20, and 200 p.p.m., respectively. All twenty-four lots were placed in storage.

2. Portions of each lot were placed in pots on greenhouse benches on April 14, April 21, May 5, July 17, and August 15. Each pot was planted with Red Kidney bean, mustard, African marigold, and barley to test by the emergence and later development of these plants the degree and persistence of toxicity from 2,4-D in the different lots of stored soils.

3. The soil originally planted on April 14 was replanted on May 12, June 2, and July 15 to test for persistence or loss of toxicity in the various treatments under cropping. Soils initially planted on April 21 and July 17 were replanted on May 19 and August 16, respectively.

4. The capacity to induce toxic symptoms was strongly persistent in soil in dry storage as measured by all initial test plantings. There was some diminution of toxicity in some lots in moist storage, although others, especially the limed ones, retained strong toxicity for 18 weeks.

5. In general, as shown by three different replantings, 4 weeks of cropping in

the greenhouse sufficed to eliminate toxicity to all test species from all soil except that which had been limed or to which 2,4-D had been added at the rate of 200 p.p.m. Seven weeks of cropping resulted in the loss of toxicity to all test species from all except the limed soil at the 200-p.p.m. level. Such long persistence of 2,4-D in this treatment suggests caution in its use in calcareous or newly limed soils. In all replantings the presence of large amounts of leaf mold strikingly reduced the degree and persistence of toxicity to one or more test species in the limed soil. Thirteen weeks of cropping resulted in complete loss of toxicity from all pots.

6. In some replantings there was more vigorous growth of seedlings in soil treated with 2,4-D than in the controls. Such stimulation was found in one or

more test species in one or more replantings for twelve of the eighteen lots treated with 2,4-D.

7. Sensitivity to 2,4-D in the soil increased in the order barley, bean, mustard, and African marigold. In the initial plantings on soil containing 2 and 20 p.p.m. of 2,4-D, the first trifoliate leaves of bean characteristically showed abnormalities involving nondivergence of the leaflets or their multiplication to numbers as high as ten.

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